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Characterization of the *in vitro* uptake of monoamines into brain microvessels

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Running title: Brain microvascular monoamine uptake

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## Abstract

The ability of brain microvessels to take up and metabolize transmitter monoamines (DA, NA, A and 5-HT) was studied in tissues from several species (rat, rabbit, guinea-pig, cat, dog, baboon, man) in vitro. Following incubation, slices were analyzed by fluorescence histochemistry and isolated microvessels by measurement of radioactivity from tritiated amine. Provided the MAO activity was inhibited a strong accumulation, similar for all amines tested, occurred in the wall (endothelial cells and pericytes) of capillaries and venules — but not in larger vessels — of all species. The degree of amine accumulation and the conditions under which it was inhibited suggested that it reflected a saturable, energy-dependent uptake, with characteristics of both the extraneuronal and neuronal type of uptake processes. The mechanism may serve as an inactivation of transmitter monoamines at the blood-brain interphase, thereby assisting in the control of transmitter levels in the cerebral extracellular compartment.





The existence of a morphological barrier between the blood and brain parenchyma is well documented (for review, see Rapoport 1976). Another aspect of the barrier function is the presence of an enzymatic blood-brain barrier for monoamine precursors, such as L-3,4-dihydroxyphenylalanine (L-DOPA) and L-5-hydroxytryptophan, first shown by Bertler et al. (1966) using fluorometric assay and fluorescence histochemical techniques. Systemically administered L-DOPA and L-5-HTP are taken up into the wall (endothelial cells and pericytes) of cerebral microvessels and is subsequently decarboxylated to the corresponding amine, i.e. dopamine (DA) and 5-hydroxytryptamine (5-HT). Using the same techniques it has been found that only minor amounts of circulating neurotransmitter monoamines, such as DA, noradrenaline (NA), adrenaline (A) and 5-HT accumulate within the endothelial cells and pericytes of cerebral microvessels (Hardebo et al. 1979a); the bulk of the monoamines is prevented from leaving the brain circulation already at the luminal membrane of the endothelial cells (Bertler et al. 1966, Hardebo et al. 1979a). Evidence has been presented for the existence for an enzymatic blood-brain barrier not only for the monoamine precursors but also for the monoamines themselves; they are rapidly broken down by monoamine oxidase (MAO) present in the endothelial cells and pericytes of the brain microvessels (Bertler et al. 1966, Spector, Baird-Lambert and Lai 1977, Hardebo et al. 1977 and 1979b) and, in addition, by MAO and catechol-O-methyl transferase (COMT) in the smooth muscle cell layer of pial vessels and brain parenchymal arterioles (Hardebo et al. 1979b). On the other hand, little is known about the clearance of neurotransmitter monoamines in direction from the brain parenchyma to the circulation. It can be assumed that the local presence of a sufficient excretory mechanism into the cerebral microcirculation — along with the reuptake into the aminergic nerve terminal and the clearance from the cerebrospinal fluid — will assist in the control of the level of the amine in the brain extracellular compartment. In the present study, a brain microvascular uptake of various neurotransmitter monoamines is demonstrated and characterized.

### Material and Methods

Animals. The study was performed on adult animals of either sex, namely 64 Sprague-Dawley rats, 29 albino rabbits, 7 guinea-pigs, 5 cats, 4 dogs, 4 baboons, and brain tissue from 5 adult female and male patients. The laboratory animals had free access to food and water. These animals were killed under Nembutal anesthesia by perfusion of the vascular system with 0.9 % saline to eliminate any error caused by the presence of blood



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#### Materials and Methods

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cells. The brains were dissected out and kept in Krebs-Ringer buffer solution on ice (for composition, see below) or, during preparation of microvessel fractions, in cold phosphate buffer. The human tissue (macroscopically intact frontal and temporal cortex) was obtained during neurosurgical lobe resection operations; the tissue was immediately placed in ice-cold buffer solution and transported on ice to the laboratory (within 30 min).

Incubation of tissue slices. Approximately 1 mm thin slices of the parietal cortex (frontal and temporal cortex in man), caudate nucleus, cerebellum, hypothalamus (at the level of the median eminence), spinal cord, and heart from the various animals were cut with a razor blade and transferred to incubation vials containing ice-cold Krebs-Ringer buffer solution. The buffer solution had the following composition (mM): NaCl 118, KCl 4.5,  $\text{CaCl}_2 \times 2\text{H}_2\text{O}$  2.5,  $\text{MgSO}_4 \times 7\text{H}_2\text{O}$  1.0,  $\text{NaHCO}_3$  25,  $\text{KH}_2\text{PO}_4$  1.0, to which was added 1 mg/ml glucose, 0.2 mg/ml ascorbic acid and 0.05 mg/ml EDTA.

The vials were placed in an incubation bath at  $37^\circ\text{C}$  for preincubation during 20 min in the presence of the MAO inhibitor, nialamide ( $10^{-3}\text{ M}$ ), followed by incubation during 20 min after the addition of various doses (1, 10 or 100  $\mu\text{g/ml}$ ) of NA, A, DA, or 5-HT. In addition, slices from the parietal cortex of rat were incubated in the presence of several increasing doses (1, 2, 5, 10, 20, 50 or 100  $\mu\text{g/ml}$ ) of NA in an attempt to find an upper limit for the uptake process. The buffer solution was continuously aerated with a mixture of 95 %  $\text{O}_2$  and 5 %  $\text{CO}_2$ , giving a pH of about 7.4 and a  $\text{pO}_2$  around 200 mm Hg (as measured on an MRK II blood gas analyzer; Radiometer, Copenhagen). Slices from the parietal cortex (frontal and temporal cortex in man) were also incubated with NA, A, DA or 5-HT (1 or 10  $\mu\text{g/ml}$ ) in the absence of nialamide. Control slices were incubated without addition of any drugs.

In order to further characterize the uptake, the incubations of the parietal cortex (frontal or temporal cortex in man) with monoamines were also performed in the presence of either cocaine ( $10^{-5}$  or  $10^{-3}\text{ M}$ ), desmethyl imipramine ( $10^{-6}$  or  $10^{-4}\text{ M}$ ), phenoxybenzamine ( $10^{-6}$  or  $10^{-5}\text{ M}$ ), oestradiol- $17\beta$  ( $10^{-6}$  or  $10^{-5}\text{ M}$ ), theophylline ( $10^{-5}\text{ M}$ ), ouabain ( $10^{-6}$  or  $10^{-5}\text{ M}$ ), or dinitrophenol ( $5 \times 10^{-5}$  or  $10^{-3}\text{ M}$ ). These compounds were added to the bath at the start of the preincubation together with nialamide. Incubation was also performed in a low  $\text{Na}^+$ /high  $\text{K}^+$  milieu (in the buffer solution KCl substituted for all NaCl) and under a combination of anoxic conditions (continuous bubbling with 95 %  $\text{N}_2$  and 5 %  $\text{CO}_2$ ) and deprivation of energy substrate (glucose), and also at  $20^\circ\text{C}$  and  $0^\circ\text{C}$ .

Fluorescence microscopy. Following the incubation, the various tissue pieces were frozen to the temperature of liquid nitrogen and further processed for fluorescence mono-





amine histochemistry according to the standard Falck-Hillarp method (Björklund, Falck and Owman 1972). The paraformaldehyde used in the histochemical procedure had previously been equilibrated with air of 70 % humidity. The formaldehyde-induced, histochemically visible fluorophores of NA, A and DA are indistinguishable in the sense that they have the same spectral characteristics and exhibit a green light under the optical conditions used, whereas 5-HT exhibits a yellow, more rapidly fading light (Björklund et al. 1972). When evaluating the changes in the fluorescence intensity after incubations during the various conditions (see below), the different preparations to be compared were always freeze-dried and further processed simultaneously.

Isolation of cerebral microvessels. Whole brains from rats and rabbits were used. After removal they were kept in an ice-cold 67 mM phosphate buffer (pH 7.4) throughout the procedure (see Hardebo et al. 1979b). The meninges, including the pial membrane and its vessels, were carefully torn off and the choroid plexuses were removed. White matter, including the whole brain stem, was dissected away. Tissue obtained from 4 - 6 rats or 2 - 3 rabbits was chopped with a razor blade, and then gently further disrupted by some 10 strokes up and down through three different 10 - 20 ml plastic syringes equipped with a nylon net (pore size 1 000  $\mu$ , 500  $\mu$  and 280  $\mu$ , respectively) glued to their open cut end. The material was subsequently homogenized by hand with a loosely fitting Teflon pestle in a smooth glass tube (0.1 mm clearance). The homogenate was washed through one nylon sieve with 225  $\mu$  pore size. The material remaining on the sieve, after extensive washing, was re-homogenized and re-sieved 3 - 4 times through the same, carefully washed sieve. The tissue passing these sieves was collected and sieved through another sieve with 75  $\mu$ m pore size. The material remaining on this sieve after washing was re-homogenized and re-sieved in the same, carefully washed sieve. The tissue fraction remaining on the last sieve, after extensive washing, consists of capillaries, venules and a few larger vessels, whereas most of the larger vessels and vessels branching off into clusters of small vessels, as well as a few small clusters of neurons and glia, remain on the 225  $\mu$ m sieve (Hardebo et al. 1979b). Neurons, glia, small microvessel segments, and subcellular fragments pass the 75  $\mu$ m sieve. The capillary fraction is pure with regard to vessels and is only contaminated with a few glial endfeet, stuck onto the vessel wall (Hardebo et al. 1977). The yield of microvessels is less than 1/1000 of the original wet weight of the grey matter. Brendel, Meezan and Carlson (1974) have shown that a mixed fraction of intracerebral vessels, prepared under similar conditions, is metabolically active.





Incubation of isolated microvessels. The microvessel fractions were transferred to incubation vials containing ice-cold Krebs-Ringer buffer solution (composition as above). The vials were placed in an incubation bath and allowed to equilibrate for 20 min at 37° C before incubation, which was started by adding  $^3\text{H}$ -NA to the solution. The tissue preparations were incubated for 15 min at a temperature of 37° C. The buffer solution was continuously aerated with 95 %  $\text{O}_2$  and 5 %  $\text{CO}_2$ . The incubation was terminated by transferring the tissue into ice-cold buffer solution. The microvessel fraction was collected by centrifugation at 4° C at 110 g and washed twice in isotope-free cold buffer for each 10 min.

In order to further characterize the uptake, microvessel fractions were also incubated in the presence of ouabain ( $10^{-5}$  M), under combined anoxia and absence of glucose, and also at 0° C.

Measurement of radioactivity. After incubation, the microvessel samples were weighed and transferred to liquid scintillation vials. They were solubilized in 0.5 ml Soluene (Packard), and liquid scintillation counting was performed in 10 ml Instagel (Packard), as were appropriate samples (25  $\mu\text{l}$ ) of the incubation medium. Quench corrections were obtained according to conventional principles.

Drugs. Dopamine hydrochloride (Sigma);  $\text{L}$ -noradrenaline bitartrate monohydrate (Sigma),  $\text{L}$ -adrenaline bitartrate (Sigma), 5-hydroxytryptamine creatinine hydrochloride (Sigma), cocaine hydrochloride (ACO), ouabain (Sigma), 2,4-dinitrophenol (Chroma), theophylline (Sigma), desmethyl imipramine (Desipramine, Geigy), phenoxybenzamine hydrochloride (Dibenzylamine; Smith, Kline and French), nialamide (Pfizer) and  $\text{L}$ -nor-epinephrine-7- $^3\text{H}$  (New England Nuclear; 9.1 Ci/mmol).

Statistics. Mean values were compared according to the Student's  $t$ -test for unpaired data.

## Results

Incubation of tissue slices. Fluorescence microscopy of the various brain regions from control slices incubated in buffer solution alone showed a dense network of delicate catecholamine-containing nerve terminals emitting a green fluorescence; in the cerebellum only a small number of isolated green-fluorescent axons were present. The parenchymal blood vessels were equipped with varying amounts of sympathetic nerves. In the heart tissue adrenergic nerves were seen in the myocardium and in association with the blood vessels. All tissues showed a slight diffuse, non-specific greenish background





fluorescence. The vessel wall proper was in all regions essentially non-fluorescent, except for the intense autofluorescence of the internal elastic membrane of larger pial arteries.

After incubation with the various monoamines an accumulation of fluorophore well above the diffuse background fluorescence was seen in the brain microvessel walls (endothelial cells and pericytes of capillaries, and small veins) in all species studied. The intensity of the general background fluorescence was also increased. No clearcut accumulation above the diffuse background fluorescence had occurred in the walls of the brain parenchymal arterioles or heart vessels. Possibly, a slight accumulation was seen in the wall of pial arteries. Inhibition of MAO by nialamide clearly enhanced the accumulation of the fluorophores in the microvessel wall, as evidenced by a stronger fluorescence intensity in relation to the background fluorescence. At an identical concentration in the incubation bath DA, NA and A all induced a similarly strong fluorescence in the microvessel wall, whereas the fluorescence induced by 5-HT was weaker. This discrepancy in fluorescence intensity is at least partly explainable by the varying fluorescence yield in the standard formaldehyde reaction (Björklund *et al.* 1972). An upper limit of fluorescence intensity in brain microvessel walls could be disclosed when the tissue slices, pretreated with nialamide, were incubated in the presence of increasing concentrations of NA: a maximum fluorescence was seen at the 20 µg/ml concentration. It has been shown in model experiments that there is a linear relationship between fluorescence intensity and the catecholamine concentration. Due to quenching of the fluorescence at high catecholamine concentrations, a further increase in the concentration above a certain level gives an unchanged or even slightly diminished intensity (Ritzén 1966, Jonsson 1971). However, our finding most probably reflects an upper limit for accumulation in the microvessel wall rather than being the result of quenching, which occurs only at very high local concentrations.

The inhibitory influence on the accumulation of NA by various drugs or by modification of the medium are listed in Fig. 1 and illustrated in Fig. 2. The pattern of inhibition was the same for the other monoamines studied.

Incubation of microvessels. An accumulation of radioactivity was obtained in the microvessel fraction after incubation in the presence of tritiated NA, as evidenced by a considerably higher radioactivity than in the incubation medium (Fig. 3). The uptake was significantly reduced by incubation at 0° C or by blockade of Na<sup>+</sup>/K<sup>+</sup>-dependent ATP:ase activity with ouabain (Fig. 3).





## Discussion

The present study provides evidence for the existence in various species including man of a saturable, energy-dependent uptake mechanism for neurotransmitter monoamines in the endothelial cells and pericytes of brain microvessels, in contrast to the endothelium of brain parenchymal arterioles. The study does not, however, show whether the uptake occurs across the luminal or the abluminal side of the microvessel wall, or both. An uptake of monoamines into brain microvessel walls of the rat has been reported by estimation of formaldehyde-induced fluorescence following incubation of brain DA, NA and their  $\alpha$ -methylated analogues (Hamberger 1967). In the latter study it was found that the uptake was prevented by desmethylinipramine, d-amphetamine and cocaine to a similar extent as the uptake into NA-storing nerve terminals of reserpine-pretreated animals.

The efficiency of the  $^3\text{H}$ -NA uptake, as reflected by the ratio of radioactivity in the microvessel fraction and the medium, was of the same order as that reported for the uptake into sympathetic nerves under similar conditions (Edvinsson and Owman 1977, Alm et al. 1979), but considerably higher than the extraneuronal uptake in smooth musculature (Alm et al. 1979). The energy-dependency of the microvessel uptake was confirmed by the inhibition accomplished by hypothermia, combined anoxia and glucose deprivation, or uncoupling of the oxidative phosphorylation with dinitrophenol. The results from the incubations with low sodium and high potassium, as well as in the presence of ouabain, suggest that the uptake is linked with a  $\text{Na}^+/\text{K}^+$ -dependent ATP:ase. The uptake was inhibited both with compounds that are known to affect the neuronal (cocaine, desipramine, phenoxybenzamine) and extraneuronal (normetanephine, oestradiol, phenoxybenzamine) transport of monoamines. This shows that, although the accumulation in brain microvessels is extraneuronal, it has several features in common with the axonal uptake process, however without the subsequent cytoplasmic retention of the amine by a reserpine-sensitive storage mechanism (Bertler et al. 1966). Thus, the accumulation of amines in the cerebral microvessels in many respects resembles the uptake shown to occur in the wall of pulmonary microvessels (for review, see Gillis 1976), though a difference in the degree of uptake between the various amines could not be established.

The extraction of trace-amounts of neurotransmitter monoamines from the brain circulation during a single capillary passage is only about 3 - 5 % (Oldendorf 1971, Hardebo et al. 1977, Hardebo and Nilsson 1979). Only at high circulating concentrations, and after inhibition of the MAO activity, is it possible by fluorescence microscopy





to visualize a weak accumulation of monoamines in the brain microvessel wall (Hardebo et al. 1979b). The poor penetrability across the luminal endothelial membrane is due to the unity of the structural blood-brain barrier (Reese and Karnovsky 1967), which to a large extent impedes the passage of these water-soluble and polar substances. On the other hand, when the blood-brain barrier is opened experimentally, an accumulation of monoamines in the cells of the microvessel wall is clearly distinguishable (Flodmark et al. 1969, Hardebo et al. 1979a). Under these conditions the monoamines may enter the cytoplasm of the endothelial cells by pinocytosis (Hansson, Johansson and Blomstrand 1975), or it may pass between (or through) the endothelial cells to reach the abluminal side of the endothelial membrane and from here enter the endothelial cell as well as the pericyte. It can be assumed that a sufficient inactivating mechanism for extraneuronal monoamines in the brain parenchyma is of fundamental value for maintaining an adequate brain function; a clearance process via the brain microvessel walls may work as a local complement to the neuronal re-uptake mechanism and enzymatic breakdown. It is therefore not unexpected that the active uptake of monoamines into the microvessel walls is exclusively working across the abluminal membrane of the endothelial cell, in direction from the brain into the cytoplasm of this cell (and of the pericyte). This would explain why monoamines, following intraparenchymal (Bertler et al. 1966) or intraventricular infusion (Fuxe and Ungerstedt 1968, and own unpublished observations), accumulate in the microvessel walls in a narrow zone around the stich channel and periventricularly, respectively.

Pretreatment with the MAO inhibitor, nialamide, enhanced the in vitro accumulation of monoamine into the endothelial cells and pericytes of the microvessel wall. This finding offers further support for the presence of MAO in these cells (Bertler et al. 1966, Spector et al. 1977, Hardebo et al. 1977 and 1979b).

The study has shown the presence of a saturable, energy-dependent transport of monoamines into brain microvessel walls. The process shares characteristics with both neuronal and extraneuronal monoamine uptake mechanisms and resembles the microvascular transport of NA and 5-HT in the lung. Once entering the microvessel wall, the monoamines are effectively metabolized by the locally present MAO. Although monoamines penetrate the luminal membrane of the cerebral microvessel only to a minor extent, MAO will provide an inactivation of those who do enter. However, the major inactivation mechanism at the level of the microvessels is accomplished by the





active transport and metabolism of amines in the direction from the brain parenchyma. Both aspects of this inactivation at the blood-brain interphase may assist in securing an adequately low level of neurotransmitter in the extracellular compartments.

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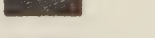
Fig. 1. Accumulation of NA fluorophore in the walls of microvessels in rat cortical slices incubated in the presence of 10  $\mu\text{g}/\text{ml}$  noradrenaline following MAO inhibition ( $10^{-3}$  M nialamide) and processed according to the formaldehyde histofluorescence method. The degree of microvascular fluorescence intensity above the diffuse, general background fluorescence is expressed arbitrarily, the tapering part of the bars indicating intermediary intensities.

Fig. 2. Fluorescence photomicrographs of brain tissue slices following incubation in the presence of catecholamine after previous inhibition of MAO ( $10^{-3}$  M in the medium). (a) - (c). Human temporal cortex. X 90. (a) Intense fluorescence in microvessel walls, but no accumulation in arterioles (arrow), after incubation with 10  $\mu\text{g}/\text{ml}$  noradrenaline. (b) In the presence of ouabain  $10^{-5}$  M the degree of noradrenaline accumulation is markedly reduced. (c) During hypothermia ( $0^{\circ}\text{C}$ ) only very little noradrenaline accumulates in the microvessel walls. (d) - (e) Rat parietal cortex. (d) Intense fluorescence in the walls of capillaries and venules (v), but not in arterioles (arrow), after incubation with 10  $\mu\text{g}/\text{ml}$  dopamine. X 110. (e) Desipramine ( $10^{-6}$  M) reduces the microvascular accumulation so that only the more voluminous nuclear region is clearly visible. X 75.

Fig. 3. Tissue/medium ratio (T/M; mg tissue per  $\mu\text{l}$  medium) following incubation of isolated cerebral microvessels in the presence of  $^3\text{H}$ -NA under control conditions ( $37^{\circ}\text{C}$ ), and in the presence of ouabain ( $10^{-5}$  M) or during hypothermia ( $0^{\circ}\text{C}$ ). Values are means + S.E.M.; number of experiments within parenthesis. Control vs. experimental according to Student's t-test: \*\*  $0.001 < p < 0.01$ ; \*\*\*  $p < 0.001$ .

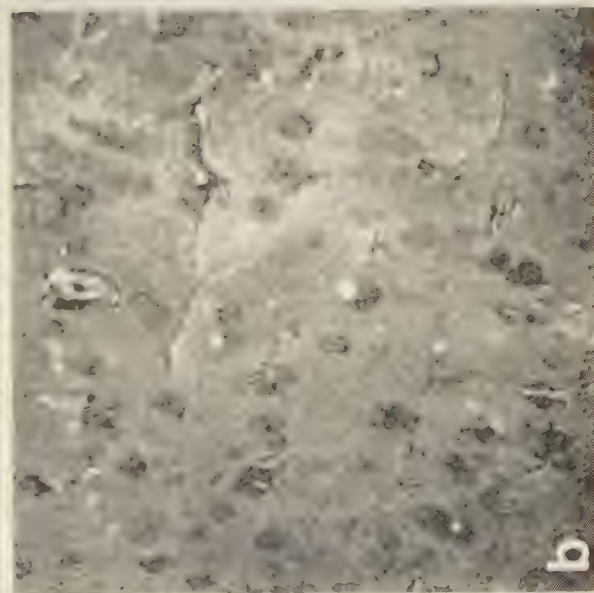




| Addition of drugs or<br>modification of medium | Degree of microvascular<br>NA accumulation<br>0 (+) + ++                             |
|------------------------------------------------|--------------------------------------------------------------------------------------|
| None                                           |    |
| Incubation at 20°C                             |    |
| Incubation at 0°C                              |    |
| Anoxia without glucose                         |    |
| Dinitrophenol $5 \times 10^{-5}$ M             |    |
| Low $\text{Na}^+$ , high $\text{K}^+$          |   |
| Ouabain $10^{-6}$ M                            |  |
| Ouabain $10^{-5}$ M                            |  |
| Theophylline $10^{-5}$ M                       |  |
| Cocaine $10^{-5}$ M                            |  |
| Desipramine $10^{-6}$ M                        |  |
| Phenoxybenzamine $10^{-6}$ M                   |  |
| Normetanephrine $10^{-6}$ M                    |  |















## References

- ALM, P., CH. OWMAN, N.-O. SJÖBERG and G. THORBERT, Uptake and metabolism of  $^3\text{H}$ -norepinephrine in myometrial adrenergic nerves of the guinea-pig: Effect of pregnancy. Am. J. Physiol. 1979. 00. 000 - 000.
- BERTLER, Å., B. FALCK, CH. OWMAN and E. ROSENGREN, The localization of monoaminergic blood-brain barrier mechanisms. Pharmacol. Rev. 1966. 18. 369-385.
- BJÖRKLUND, A., B. FALCK and CH. OWMAN, Fluorescence microscopic and microspectrofluorometric techniques for the cellular localization and characterization of biogenic amines. In: S.A. Berson (ed.) Methods of Investigative and Diagnostic Endocrinology, Vol. 1, J.E. Rall and I.J. Kopin (eds.) The Thyroid and Biogenic Amines. North Holland, Amsterdam. 1972. p. 318 - 368.
- BRENDEL, K., E. MEEZAN and E.C. CARLSON, Isolated brain microvessels: A purified, metabolically active preparation from bovine cerebral cortex. Science. 1974. 185. 953 - 955.
- EDVINSSON, L. and CH. OWMAN, Characterization of the uptake of  $^3\text{H}$ -noradrenaline into rabbit brain vessels and its relation to the degree of sympathetic nerve supply. In: Ch. Owman and L. Edvinsson (eds.) Neurogenic Control of the Brain Circulation. Pergamon Press, Oxford. 1977. p. 121 - 132.
- FLODMARK, S., A. HAMBERGER, B. HAMBERGER and O. STEINWALL, Concurrent registration of EEG responses, catecholamine uptake and trypan blue staining in chemical blood-brain barrier damage. Acta neuropath. (Berl.). 1969. 12. 16 - 22.
- FUXE, K. and K. UNGERSTEDT, Histochemical studies on the distribution of catecholamines and 5-hydroxytryptamine after intraventricular injections. Histochemie. 1968. 13. 16 - 28.
- GILLIS, C.N., Extraneuronal transport of noradrenaline in the lung. In: D.M. Paton (ed.) The Mechanism of Neuronal and Extraneuronal Transport of Catecholamines. Raven Press, New York. 1976. p. 281 - 297.
- HAMBERGER, B., Reserpine-resistant uptake of catecholamines in isolated tissues of the rat. A histochemical study. Acta physiol scand. 1967. Suppl. 295. 1 - 56.
- HANSSON, H.-A., B. JOHANSSON and CH. BLOMSTRAND, Ultrastructural studies on cerebrovascular permeability in acute hypertension. Acta neuropath. (Berl.) 1975: 32. 187 - 198.
- HARDEBO, J.E., L. EDVINSSON, P.C. EMSON and CH. OWMAN, Isolated brain microvessels: Enzymes related to adrenergic and cholinergic functions. In: Ch. Owman and L. Edvinsson (eds.) Neurogenic Control of the Brain Circulation.





Pergamon Press, Oxford. 1977. p. 105 - 113.

HARDEBO, J.E., L. EDVINSSON, E.T. MACKENZIE and CH. OWMAN, Regional brain uptake of noradrenaline following mechanical or osmotic opening of the blood-brain barrier. Acta physiol. scand. 1977. 101. 342 - 350.

HARDEBO, J.E., L. EDVINSSON, E.T. MACKENZIE and CH. OWMAN, Histo-fluorescence study on monoamine entry into the brain before and after opening of the blood-brain barrier by various mechanisms. Acta neuropath. (Berl.). 1979a. 00. 000 - 000.

HARDEBO, J.E., B. FALCK, CH. OWMAN and E. ROSENGREN, Quantitative measurement of the formation and degradation of neurotransmitter monoamines at the blood-brain interphase in various species including man. J. Neurochem. 1979b. 00. 000 - 000.

HARDEBO, J.E. and B. NILSSON, A critical evaluation of the Oldendorf method for measuring brain uptake index. Acta physiol. scand. 1979. 00. 000 - 000.

JONSSON, G., Quantitation of fluorescence of biogenic amines demonstrated with the formaldehyde fluorescence method. Prog. Histochem. Cytochem. 1971. 2. 299 - 334.

OLDENDORF, W.H., Brain uptake of radiolabelled amino acids, amines and hexoses after arterial injection. Am. J. Physiol. 1971. 221. 1629 - 1638.

RAPOPORT, S.I., Blood-Brain Barrier in Physiology and Medicine. Raven Press, New York. 1976.

REESE, T.S. and M.J. KARNOVSKY, Fine structural localization of a blood-brain barrier to exogenous peroxidase. J. Cell. Biol. 1967. 34. 207 - 217.

RITZÉN, M., Quantitative fluorescence microspectrophotometry of catecholamine-formaldehyde products. Exp. Cell Res. 1966. 44. 505 - 520.

SPECTOR, S., J. BAIRD-LAMBERT and F.M. LAI, Disposition of norepinephrine in isolated brain microvessels. In: Ch. Owman and L. Edvinsson (eds.) Neurogenic Control of the Brain Circulation. Pergamon Press, Oxford. 1977. p. 115 - 120.

